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The blood and the blood-forming organs of the monkey.

by P. Cohrs, R. Jaffe and H. Meessen (editors).

Partially translated from: The pathology of laboratory animals, Springer, Berlin 1958, p. 221-228, 271-274.

VI. The monkey.

a) Introduction.

Rhesus monkeys are utilized most frequently. Most of the data contained in the literature therefore concerns this species. Only a few authors report on studies of other types. Van den Berghe and Blitstein investigated the largest material. Of the 83 monkeys examined by them, 30 were Rhesus monkeys, 21 Cercopithecus (long-tailed monkeys), 12 Cynocephalus (baboons), 10 Cercocebus (capucin monkeys), 8 chimpanzees and 2 orang-utans. Since they inferred from careful statistical evaluation of their results that the morphology of blood cells is identical among the various simian species and that no statistically supportable differences exist in the cell count (with the exception of platelets), it seems proper to consider the following elucidations valid for all monkeys. The range of fluctuation, especially in leukocytes, is quite extensive, however. For this reason some authors list modal values in addition to extreme ones (see Table 17). It is stressed by many that simian blood has the greatest similarity to that of man (with the exception of basophilic leukocytes).

b) Procurement of blood.

Small specimens may be taken from the rim of the ear or the finger pad, just as from humans. Animals on particularly good terms with their keeper may permit puncturing in his presence without restraining measures. In general, precautions must be taken against scratching and biting. This is accomplished best by grasping both upper arms and holding them together behind the animal's back. If its breast is now pressed against a table top, the animal is helpless. A larger amount of blood may be taken from a vein. Two assistants are required who grasp the monkey's arms and legs and spread-eagle it on a table, with its back against the table. The cubital vein in the elbow bend is not easily accessible; the vena saphena, leading along the median side of the lower leg and close above the ankle, is better suited for this purpose. It emerges clearly when the lower leg is manually compressed below the knee. Narcosis is unnecessary, but may easily be imposed by inhalation of ether.

1. Normal findings.

General.

The great range of fluctuation in simian blood counts suggests an inquiry into its causes. It has been stated in the introduction that the difference in species cannot be held responsible for this circumstance. The fluctuation within the same species is as great as among different types. Differing conditions of maintenance and diet, climate and season could be assumed to be influential. However, even monkeys kept under identical conditions show great individual divergence in blood counts. It is certain, however, that the variable irritability of the monkeys plays a certain role, since no withdrawal of blood proceeds without agitation on the animal's part. According to Fox, the results gain in uncertainty with the frequency of puncture.

All investigators agree that no dietary effects on the blood count can be established, with the exception of the genuine deficiency diseases that are usually caused experimentally. Klieneberger, particularly, denies the existence of digestive leukocytosis. Fox tried to establish possible daily fluctuations by obtaining specimens every 4 hours. He found a regular rhythm only in leukocytes, predominantly in neutrophils. Their number was lowest at noon and nearly doubled toward midnight. Simultaneous measurements of body temperature showed an opposite course, being highest at noon and 4°F (= 2.2°C) lower toward midnight.

Little is known about differences in age. On the other hand, sexual dissimilarity has been investigated by van den Berghe and Blitstain, who examined 29 males and 35 females of 4 different simian species. They found the erythrocyte count in males to be slightly higher than in females; leukocytes were reversed; hemoglobin readings were identical. The percentual distribution of cells in the leukocytic series revealed irregular differences, they could not be confirmed statistically.

a) Red blood cells.

As shown in Table 17, the fluctuation range of erythrocytes reaches extremes of 3.6 - 7.9 million. The most frequent values lie between 4.5 and 6 million. The higher values suggest that cells may have been obtained from blood storage during puncture due to the animals' refractoriness. The erythrocytes have a median diameter of 7.5 microns, but there is distinct anisocytosis; the lower extreme is 3.5 microns and the upper 8.5 microns. Jagged, crescent-shaped forms are frequent. Polychromatic erythrocytes are always present, though in small numbers. Erythroblasts are rare.

Personal examinations revealed 0.3 - 0.7% reticulocytes among the erythrocytes. As evident from Table 17, these figures agree fairly well with those of other researchers.

b) Hemoglobin.

The values listed in Table 17 are to be compared with reservations only, as far as the Sahli units are concerned, since the information regarding the hemometers utilized is not always clear. According to personal observations the Hb content fluctuates between 65 and 99 with a median value of 75 Sahli units; this corresponds to a content of 10.4 - 15.8 g and a median value of 12.0 g per 100 cm^3. The table shows that this agrees with the data of most other authors. A few values that greatly exceed this limit may surpass the normal range.

Klieneberger as well as van den Berghe and Blitstein furnish data on the color index. They have used the computation customary with human blood and for this reason arrived at a normal value below 1.0, conforming to the lower Hb content of simian blood. Klieneberger lists the C.I. at 0.6 - 0.7, van den Berghe and Blitstein give 0.8 - 0.9 as a modal value. Schermer has proposed the computation of the simian C.I. by means of the following formula:

$$\frac{Hb}{E} \times \frac{5}{75} = \text{color index.}$$

This computation yields a color index of 1.0 as normal value.

c) White blood cells.

General.

The extraordinarily great range of fluctuation in leukocytes has already been pointed out. When van den Berghe and Blitstein list one of 1,300 - 40,000, this should far exceed the limits of normal values in both directions. It may be assumed that pathological findings have been included or that methodic errors are involved. Most of the data of other authors (see Table 18) are within much narrower limits. Yet these are still far apart at 3,700 (Klieneberger) as the lowest and 34,700 (Shukers) as the highest value, and the establishment of a modal value incorporating 2/3 of all data becomes a necessity (see Hall, Shukers, van den Berghe in Table 18). The median value computed by the individual authors do not reveal quite as large a divergence. They range from 7,400 (Klieneberger) to 18,100 (Bilimoria); most of them are near 14,000. If consideration is given to the fact that Fox has determined daily fluctuations by twice that amount in the same animal (10,000 and 20,000, respectively), the conclusion is inescapable that the monkey is extremely labile with respect to the leukocyte count and that great care must be exercised in the evaluation of possible changes.

Neutrophilic leukocytes.

The percentual share of the various leukocytes also shows considerable fluctuation, but the median value usually is around 40%. This means that the number of neutrophils normally is slightly lower than that of lymphocytes. According to Schermer, the neutrophils have a diameter of 12-14 microns and a strongly pycnotic, intertwined, sinuous and constricted nucleus that frequently is partitioned into several segments, at times connected by fine threads. Super-segmentation into up to 10-12 individual segments has also been observed. The protoplasm is densely filled with very delicate, rod-like, pale red granules (see Fig. 13 a). Some animals reveal staff cells which may amount to 2% of the neutrophils.

Eosinophilic leukocytes.

Their number varies, some animals fail to show any, others have them in abundance. Most authors found a median value of 2-3%. Where greater numbers were found, the authors in question assume a possible infestation with worms.

The differentiation of eosinophils and neutrophils may be difficult since they are of equal size and the latter also possess red granulation. However, the granules of the eosinophils are at least twice as large and always round, not rod-like as in the neutrophils; they also stain more intensely. The background of protoplasm is grayish-blue and much darker in tone than in the neutrophils. The nucleus is less pycnotic, shows horseshoe, ring and S forms, and often parts into 3 segments (see Fig. 13 b).

Basophilic leukocytes.

Table 18 reveals that basophils may be entirely absent at times and that they may reach a maximum of 3% (Shukers). They are as large as neutrophils, some are even larger. The broad horseshoe or ring-shaped nucleus is barely pycnotic, shows only slight constrictions and occupies the major part of the cell. The protoplasm has an irregularly stained, slate-gray basic hue and, according to Schermer, is filled with two different kinds of granules. One type is composed of pale, gray-colored rods, closely packed and occupying the entire protoplasm. In addition, there are isolated to numerous very intensely stained, dark violet, round to rod-shaped granules of very variable size (see Fig. 13 c). Only the latter conforms with real basophilic granulation. Schermer was unable to discover transitory stages of these two types of granules. Hall emphasizes that basophils are the only blood cells with a distinct difference in man and monkey. He considers simian basophils most similar to those of the guinea pig. According to personal observation, this similarity is not too striking, it consists primarily of the fact that the granules of both animals are much larger than in man (cf. Fig. 9 c).

Lymphocytes.

The lymphocyte is the most common cell in simian blood. According to the data of most authors (see Table 18), they reach or surpass 50%. Only Klieneberger undertook a differentiation of large and small lymphocytes, he found 12% large ones and 46% small ones. The lymphocytes do not differ from those of man. They are slightly larger than erythrocytes, have a round, at times somewhat indented, coarsely lumped, pycnotic nucleus and basophilic protoplasm that becomes lighter toward the nucleus. The larger lymphocytes at times reveal numerous very fine azure granules. The frequent occurrence of twin nuclei in one cell is a special peculiarity.

Monocytes.

The differentiation of monocytes from the large lymphocytes is not always easy, and sometimes impossible. Hall even claims to have seen transitions between these two and makes this the basis of his assumption that monocytes develop from lymphocytes. The existence of uncertainties is indicated by the percentages listed by the various authors. When 15% monocytes are indicated, this surely must include some lymphocytes. In reality their number will rarely exceed 3%. For purposes of identification, human hemal morphology may be used as an informal guide. Simian monocytes also have a large, barely pycnotic, light nucleus that is seldom round, usually indented or lobulated. The protoplasm is wider than that of the lymphocytes and has a grayish-blue, cloudy hue. Aside from these monocytes, typical for all mammals, Schermer found another type which he called endothelial monocytes and which, according to Heilmeyer, occasionally occur also in man. They have a large, oval nucleus without any kind of indentation, but frequently show a distinct, light nucleolus, the chromatin is finely granular and lighter than the coarse structure of lymphocytes. The protoplasm is very wide, grayish-blue, finely granular, becomes lighter toward the edge and here presents an extremely fine, reddish granulation. At times the edge appears to be jagged.

Miscellaneous blood cells.

Klieneberger, Hall and Schermer report on the isolated occurrence of typical plasma cells in the peripheral blood. Fox also found occasional neutrophilic myelocytes.

Platelets.

The data of the various authors (see Table 17) concerning the platelet count deviate to a high degree. We note 32,000 as the lowest and 1,550,000 as the highest value. It is only with respect to thrombocytes that van den Berghe and Blitstein claim to have found statistically supported racial differences. The lowest average values

are shown by chimpanzees, capucin monkeys and long-tailed monkeys (221,000 - 239,000), the highest by baboons (356,000) and Rhesus (506,000). We agree with the investigators on the chance of grave error in the counting of platelets, in view of their coalescence.

The platelets present the usual appearance: Heavy violet granulation in a hyaline, pale bluish background.

d) Miscellaneous biological investigations.

Amount of blood.

The absolute amount of blood in monkeys is 4.35 - 9.02% of the body weight, according to Schulz and von Krueger. Schermer was able to collect less than half of this amount, i.e. 1/30 or 1/33 of the body weight by bleeding from the carotis.

Cell volume.

Shukers and coworkers used the hematocrit method on Rhesus monkeys and obtained a cell volume of 31.8 - 49.0% with a median value of 40.0 plus-minus 0.35.

Sedimentation rate.

Westergreen's method gave the following results:

Authors	After 1 hr.	2 hrs.	24 hrs.
Nohlen and Sarvan	1.7 - 3.5	2.1 - 9.2	17.6-56.7 mm
Schermer	1.2	2.5	19 mm

Coagulation time.

Schermer lists 2 min 5 sec as the start of coagulation. Shukers and coworkers used Kolmer's coagulometer and established a coagulation time of 19-239 sec, with a median at 78 plus-minus 46 sec.

Resistance of erythrocytes.

According to Schermer, hemolysis starts at 0.4%, is complete at 0.3% NaCl.

Electrophoretic examination
of plasma and serum.

Deutsch and Goodloe examined the plasma of 3 Rhesus monkeys by Tiselius' method and obtained:

Albumin	α_1-	α_2-	α_3-	β-	φ (fibrinogen)	γ-
	globulins					
50±0.5	5.9±1.0	5.2±0.5	4.7±0.5	16.1±0.9	8.4±0	9.0±0.4

Moore also tested the serum of 22 Rhesus monkeys under the following conditions: pH 7.6, phosphate-NaCl buffer, ionic strength 0.2, dilution 1:4. He divides the appearance of electrophoresis into 6 components and lists the following values (the median values in parentheses):

Component 1	2	3	4	5	6
47-62(52)	0	3-11(8)	13-20(16)	0	14-29(22)%

Schermer reports on the results of 2 tests (Antweiler's method, pH 8.6, veronal - veronal sodium buffer with ionic strength 0.12):

	years	albumin	α_1-	α-	α_2-	β-	γ-
					globulins		
1 Rhesus monkey	1½	25.1		14.5		38.6	21.8%
1 Rhesus monkey	4	23.7	9.2		8.7	27.2	31.2%

Since the test methods utilized are not uniform, the resulting values cannot very well be compared.

Pathological findings.

Since the monkey is the animal closest to man, it could be expected that human blood diseases will be found also in the monkey. Actually, almost nothing is known about this situation. A few data are known only about the leukemias and various parasitic diseases, especially plasmodias, babesias and trypanosomes. Since these are to be covered in a separate chapter of this book, only the barest reference will be made thereto at this time.

(page 271).

g) Monkey.

α) Technique of bone marrow procurement.

Sternal puncture is recommended as a means of procuring bone marrow. The technique is the same as that described by various authors with respect to humans. It must be noted, however, that the Rhesus monkey has only one sternal segment wide enough for puncture: the segment located between the clavicles (Schermer 1954). We found

farther caudally situated segments in baboons and capucin monkeys that permit successful puncture. In addition, trepanation or puncture of one or more ribs is feasible. Examination of femoral marrow, as is customary with other animals, may also be performed. It is understood that narcosis is mandatory in the bone puncture of monkeys.

β) Distribution and development of bone marrow.

Stasney and Higgins (1936) examined the bone marrow of 3 monkeys (Macacus rhesus) macroscopically. There was no fatty marrow in the sternum, rib and vertebra, only red marrow. The center of the femoral and tibial shaft contained fatty marrow, socalled yellow marrow. Two younger chimpanzees showed only red marrow in the femur. In contrast to the macroscopic appearance, no great differences in the cellular content of the examined regions were noted microscopically by Stasney and Higgins (1936) and Schermer (1954). No differences were noted either between the marrow of the sternum, ribs and vertebrae, or in the cellular content of the femoral and tibial marrow. Significant divergences were found only upon comparison of the cell content of flat bone marrow (sternum) with a tubal bone (e.g. tibia). They involve only the socalled leukoblasts (hemocytoblast?), myelocytes, metamyelocytes and normoblasts. Leukoblasts, myelocytes and metamyelocytes are less numerous in the femur and tibia than in the sternum, rib and vertebra. The content of normoblasts, on the other hand, is greater in the long bones than in the flat ones. The preponderance of erythropoiesis in the tibia is noteworthy to the extent that yellow marrow, i.e. fatty marrow, is found here. Fig. 18 depicts the numerical results graphically.

Taliaferro and Mulligan (1937), in connection with their studies of Macacus rhesus and Macacus cynomolgus, emphasize the great differences between various animals. They list the occurrence of typical lymph follicles as a peculiarity of simian bone marrow. The lymph follicles resemble those of the spleen. They contain medium and large lymphocytes with numerous mitoses. According to Taliaferro and Mulligan (1937), lymph follicles are rare in Panamanian monkeys. Whenever they occur, they are invariably accompanied by an intensive malarial infection. It is questionable whether the occurrence of lymph follicles, which is not mentioned elsewhere, can be considered a normal bone marrow condition of the monkey. The presence of lymph follicles is rare in all other mammals, including man.

I have been unable to find precise data on the development of simian bone marrow. It should follow the course known from other mammals. Suarez, Diaz-Rivera and Hernadez-Morales (1943) have studied the change in the composition of the cellular picture in Rhesus monkeys by means of 4 young, 8 adolescents and 28 adults. The higher degree of erythropoiesis found in young monkeys yields to myelopoiesis in the adult. Within myelopoiesis and erythropoiesis, the more mature elements also occupy the foreground in the adult. Details are given in Table 16.

γ) Morphology and composition of bone marrow.

The hemocytoblast has a light nucleus and 1-2 nucleoli. The cytoplasm is light and wide. Advancing maturation reduces the nuclei of promyelocytes, myelocytes and metamyelocytes in size, they become more indented and gain in chromatin. Nucleoli appear up to the myelocyte. The cytoplasm originally stains pale blue, later red. The neutrophilic granulation is pale pink in the promyelocyte, in part also in the myelocyte. However, dark violet granules are also seen. The latter are absent in metamyelocytes, staff cells and segmented cells. Segmented cells are rare in the bone marrow. The granulation of eosinophilic granulocytes is a vivid yellowish-red, that of basophils is grayish-blue.

The erythropoietic series offers no peculiarities. The orthochromatic erythroblasts readily divide into 2-3 nuclear remnants, forming a clover-like structure. Plasma cells are abundantly represented (Schermer 1954). The cytoplasm is deep blue and equipped with vacuoles. According to Schermer (1954), the megakaryocytes have a finely granular, violet cytoplasm containing 3-5 individual nuclei.

Incidentally, Stasney and Higgins (1936) stress the similarity of simian bone marrow cells with those of man, dog and rat and for this reason deem a description of the morphology superfluous.

The supply of erythrocytes is reported to be 5.5 million for Rhesus monkeys, without the considerations of age and sex (Suarez, Diaz-Rivera and Hernadez-Morales, 1945). Leukocytes number 16,210, the erythropoiesis-granulopoiesis index is said to decrease with age. Stasney and Higgins (1936) state that the erythropoiesis-granulopoiesis index is considerably greater than 1 in the flat bones, slightly greater in the femur and less than 1 in the tibia. Table 17 shows the ratio of myeloid, erythroid and lymphocytic cells in 5 localizations and the erythropoiesis-granulopoiesis index of the same points. Table 18 lists the results of bone marrow counts at different locales by way of comparison, while Table 16 reflects the composition of bone marrow at different age levels. The knowledge of these conditions deserves special attention in studies of bone marrow. Unfortunately the results of different authors cannot be compared with facility, as is true also of other laboratory animals. A critical exposition must needs be omitted.

Figures and tables (second part).

Fig. 16. Simian bone marrow cells in different locales (according to Stasney and Higgins, 1936). (The numbers on the bottom correspond to the cells listed in Table 18).

Table 16. Composition of bone marrow cells in rhesus monkeys at various age levels (according to Suarez, Diaz-Rivera and Hernadez-Morales, 1942). 4 young, 8 adolescents and 28 adults.

Table 17. Frequency of the most important marrow cells and granulopoiesis-erythropoiesis index in 5 different bone marrow regions (according to Stasney and Higgins, [illegible])

Table 18. Distribution and frequency of bone marrow cells in the rhesus monkey at 5 different bone marrow locales (according to Stasney and Higgins, 1936).